05/25/2005

EPA (OPPT, NERL, NRMRL) Comments on Noack Laboratory Draft Standard Study Plan *Aerobic Transformation in Soil (Version 2004-12-28)*

Section Number	Comment
3.6 Further Information	What is the purpose for including information on analytes not monitored in the study? *This has been modified in the protocol.*
	Unclear from language under 3.6 which analytes in Table 3 will or will not be monitored. Suggest clarification in table. *This has been modified in the protocol.*
5. Test System Test concentration	

Description of the Test System	The use of one soil is proposed. As stated in the OECD 307 guideline, one representative soil can be used if the goal is to determine transformation pathways. If the goal is to determine transformation rates "at least three additional soils" should be used. The proposed use of a single soil will not adequately allow the determination of transformation rates. A minimum of four soils should be included. *A single soil has been selected because the initial test plan will look for potential transformation products, pathways, and the ability of the test system to provide data needed to meet the objectives of the test (the ability of the test to detect polymer degradation). A single soil is recommended in the guidelines (paragraph 23) to determine transformation pathway.* *Additionally, TRP expects that the transformation rates may be so long (e.g., half-lives greater than 500 years) that the transformation rates for different soils would be essentially the same within the experimental error of the analytical methods. However, if testing demonstrates that the half-life is less than 500 years, TRP will consider additional testing.* *Furthermore, the TRP plan should be viewed as method development so that the laboratory and individual TRP companies can develop collective experience in conducting this type of test. The testing proposed by the TRP, however, does not prevent other TRP companies from conducting additional tests on additional test items or in additional soils. It should also be pointed out that the TRP is conducting and planning tests in other media, including aerobic sludge, anaerobic sludge, and sediments.*
	Will acclimated or naïve (no prior exposure to fluorotelomers, and co-contaminants, or degradation products) soil be used? *The draft protocol does not include an acclimation phase or the use of soils that are known to have been previously exposed to these compounds.*
Soil Handling	Preincubation of 2 – 28 days is conducted to reestablish the equilibrium of microbial metabolism. How is the duration of preincubation determined? *Based on experience, 10 to 14 days are sufficient to reestablish the equilibrium of the soil microbes. Metabolism of a reference compound (glucose) will be checked during the course of the experiment (see below).*

6 Test Groups Control	How does control differ from Positive Control *The control is untreated active soil that will be extracted and analyzed for specific analytes. The positive control is untreated active soil to check the activity of the microbial biomass at each sampling time by adding a readily biodegradable substance or checking microbial biomass, for example.*
Test Chemical Concentration	1000 mg/ kg dry weight is proposed. This concentration can be increased so long as adverse impact on soil microorganisms is not observed. Starting concentrations should be chosen considering the ability of analytical methods to detect low levels of transformation of the test substance. This can be based on experience from other biodegradation testing of similar substances. *The concentrations at which these test items may occur in soils are expected to be very low in landfills and agricultural soils to which sewage sludge is applied compared to the level proposed in the test. Criteria for applying them in the test, however, will be the highest concentration tested in a respiration inhibition study shown to be non- inhibitory and does not affect soil Water Holding Capacity, texture, pH, microbial biomass, or no greater than 10,000 mg / kg soil dry weigh. For example, current analytical methods have been demonstrated capable of measuring the analytes of interest in the sub-ppm (µg/kg) levels. If one assumes that the polymers contain no less than 10% (wt. basis) 8-2 OH covalently bound to the polymer and if 1% of 10,000 mg polymer/kg soil were to degrade, this would result in an equivalent of 10 mg 8-2 OH/Kg released to the soil (or 10,000 µg/Kg) if 10,000 mg polymer/Kg is the application rate. The LOQ for the 8-2 OH based on current technology is estimated at ~10 µg 8-2 OH/Kg. For 8-2 COOH, 8-2 U COOH, and 8-COOH the LOQ is also estimated at 10 µg/Kg. This indicates that degradation of 1% of the polymer will, at a minimum, result in a concentration of the 8-2 OH and the acids of 1000 times their respective LOQs.*
Abiotic Test Item Control	How will the soils be sterilized? Will they check samples of the soil for microbial activity following sterilization? *Soils will be irradiated and then treated with chemicals to maintain sterility. The study will include a check on sterility, such as adding glucose to the sterile control soils and checking mineralization by determining CO_2 (aerobic systems) or CH_4 (anaerobic systems) and by using*

	a standard microbial plate count method.
7 Method Test Vessels	While the exact volume of soil sample used may not be known ahead of time, can a range estimate for the mass/volume of soil sample desired in each reactor be provided? *A range of soil weight (20 to 30 g) has been added to soil test protocols.*
Replicates	Are duplicates enough for statistical comparison between reactor types? How many samples from each vessel will be analyzed at one time period? *Because of the numbers of sample time points and the expectations that there will not be any significant ($p = 0.05$) change in analyte concentration by time, there should be sufficient replicates when the results are combined by time. On the other hand, significant polymer degradation (greater than 1%) will result in an increase in the sum of 8-2 OH and acids of approximately 22 µM/kg compared to an LOQ of ~0.02 µM/Kg for 8-2 OH and for the acids.* *The contents of the entire vessel will be extracted for analysis after a headspace sample is collected.*
Aeration	How will the flasks be aerated? I would like to see a schematic of the proposed reactor system. It would help to clarify the experimental design. *A picture is provided for the static system. A static system will be used in the aerobic and anaerobic tests so that aeration will not be necessary. Oxygen content in the headspace (aerobic system only) will be checked periodically. If O_2 is less than 19%, a headspace sample will be collected, the vessel opened to the air for a minimum of one minute and then recapped.*
Application	What does "mixed with an appropriate part of the soil" mean. More information concerning the test items introduction into the soil sample is necessary. *The test substance will be mixed into the entire soil sample in the test vessel to minimize steps and potential loss of test material.*
	If resuspended test substance is used include a negative control dosed with emulsifiers and solvents used to resuspend test substance but not dosed with test substance. *Solid polymer as a powder will be used in the test and it will not be redispersed or resuspended after purification.*

	Recommend keeping the mass of soil constant in all vessels as soil mass to air ratio may be an important variable. *Except for small changes as a result of adding the test polymer or spikes, this will be done.*
Distribution Uniformity	Method for evaluating homogeneous distribution of polymer is unclear. Will three flasks or three subsamples from a single flask be evaluated? Action to be taken in the event that distribution criteria are not met is unclear. *Since test item will be added to each test vessel and the entire contents of each vessel will be extracted, evaluating homogenous distribution will not be necessary.*
7.1 Performance of Study Course of Study	Will soil samples be mixed during the experiment? *No further mixing of the soil is planned other than at the start of the study.*
	How much soil will be transferred? *No soil will be transferred. Rather, the extracting solution will be added to the test vessel with soil after a headspace sample is collected, recapped, and mixed.*
	What is the acceptable range for soil moisture losses? *Soil moisture will be maintained between 40 and 60% Water Holding Capacity.*
	Will reactor location be randomized? *No advantage is seen in randomizing all the test vessels, although there may be value in a stratified random sampling design, vessels randomized within each time point, for storage. The test vessels will be stored in boxes in the dark in a constant temperature room. A randomization process to dose and to sample the test vessels may result in further handling of all samples and complication of the set-up, sampling, and analyses that could increase the opportunities for errors.*
7.2 Types and Frequency of Measurements	It is recommended that measurements include pH, water holding capacity, and CO_2 or O_2. *The tests will be conducted following the OECD test guidelines. Soil pH will be determined at the start of the test and during and at the end of the test in the anaerobic systems. WHC and O_2 (aerobic system) or CH_4 (anaerobic system,) and other parameters will be monitored during*

	the course of the study.
Sampling Schedule	Will samples be analyzed immediately? If held prior to analysis, sample stability should be determined. *Samples will be extracted immediately. Extract concentrations will be determined within 24 hours or frozen until analyzed. Storage stability will be determined on frozen extracts.*
Type of Determinations	How will glucose induced respiration rate data be used? *Microbial biomass will be used to help determine the activity of the soil microorganisms.*
8 Specific Analysis	What are the expected MDL and MQL for the LC-MS/MS and GC-MS methods? They should be sensitive enough to detect/quantify possible low levels of transformation of the test substance. *Please see previous responses. The LOQ (MQL) for the 8-2 OH and acids based on current technology is estimated at ~10 µg /Kg. The LOD (MDL) should range from about 1 to 3 µg/Kg. The LOQ and LOD will be estimated as part of the method development/validation based on the signal to noise.*
	Information on quality control methods, data acceptance criteria, and corrective action for all analysis should be provided. *This is being done as part of the method validation for this analyte.*
Demonstration of analytical capability	Can a recovery demonstration of 8-2 OH alone be conducted? *This is being done as part of the method validation for this analyte.*

8.1 LC-MS/MS Analysis	Background instrument contamination is a large problem when analyzing these compounds. What is being done to address this issue? Also, how is the LOQ going to be determined? *There is an SOP for checking controls and for cleaning the laboratory equipment and analytical instruments to address and monitor background and to address cross-contamination. This SOP has been provided to the EPA. This is no longer viewed as a concern.* *The LOQ and LOD will be estimated as part of the method development/validation based on the signal to noise ratio.*
	Can complete analytical details be provided in this document? *The analytical methods are underdevelopment and will be reported in a document separate from the protocol or as an amendment to the protocol.*
8.2 GC-MS Analysis Extraction Method	Will we be able to review this amendment prior to accepting the final work plan? *Yes, as long as this review does not affect the progress of the study. Acceptance of an amendment is usually reserved for the study director after notification of the study monitor, especially for significant amendments to the protocol.*
9 Evaluations Statistical Methods	Describe statistical analysis methods to be used to analyze results. *Data will be transformed from weight concentrations to molar concentrations using a computer program, such as Microsoft EXCEL®. Data will be assessed for normal distribution and transformed, when necessary, prior to performing statistical techniques, such as means, standards deviation, correlations, etc.*
10 Validity of Study Validity Criteria	What general validity criteria have been used by the Dr U Noack - Laboratorien for other similar studies performed in the past? *There is no validity criteria stated in the guidelines as long as the guidelines are followed for parameters such as WHC, microbial biomass, recoveries, etc. TRP has discussed potential criteria for these studies with the contract lab but has decided to keep them out of the protocol.*

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